UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

November 10, 2008

MEMORANDUM

Subject: Efficacy Review for Cryocide 20, EPA Reg. No. 9150-11; DP Barcode: D355411

From: Ibrahim Laniyan, Microbiologist
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11/13/08

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To: Emilie Mitchell / Wanda Henson
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Applicant: International Dioxide, Inc.
554 Ten Rod Road
North Kingstown, RI 02852

Formulation from the Label:

Active Ingredient	% by wt.
Chlorine Dioxide	0.72 %
Didecyl Dimethyl Ammonium Chloride	0.40 %
Other Ingredients	98.88 %
Total	100.00 %

I. BACKGROUND

The product, Cryocide® 20 (EPA Reg. No. 9150-11), is an EPA-approved hospital grade disinfectant with virucidal and tuberculocidal activities. The applicant requested an amendment to the registration of this product to add claims for killing mold and mildew / *Aspergillus niger* by submitting efficacy data generated using the product, Cryocide® Disinfecting Spray (EPA Reg. No. 71654-22), an EPA-approved broad spectrum / general use disinfectant. Study was conducted at MicroBioTest, Inc., located at 105B Carpenter Drive in Sterling, VA 20164.

This data package identified as D353302 contained a letter from the applicant to the Agency (dated July 15, 2008), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-35 (Data Matrix), **one study (with MRID Nos. 474275-01 and 474844-05),** Statements of No Data Confidentiality for the study, and the proposed label (dated 11/04/08)

II. USE DIRECTIONS

The product is designed for use in disinfecting hard, non-porous surfaces (such as bath and shower stalls, sinks, counters, toilets, hampers, diaper pails, appliances, basins, bathroom fixtures, bathtubs, cages, chairs, countertops, floors, tables, urinals and walls) in bathrooms and kitchens and for controlling mold and mildew growth. The label indicates the product may be used on surfaces composed of fiberglass, formica, glass, ceramic, metal, plastic, painted surfaces, and steel. Directions on the proposed label provided the following information regarding preparation and use of the product:

> For General Disinfection: Use undiluted (neat). Apply product to hard, non-porous surfaces, thoroughly wetting surfaces with a cloth, mop, sponge or sprayer, or by immersion. Treated surfaces must remain wet for 10 minutes. Wipe dry with a cloth, sponge or mop or allow to air dry. For heavily soiled surfaces, a pre-cleaning is recommended.
>
> Mold and Mildew Control: Dilute 1 part of Cryocide® 20 into a clean pail and add 4 parts water to make up working solution. Spray 6-8 inches away from a dry, pre-cleaned surface until thoroughly wet. The treated area must remain wet for at least 10 minutes. Allow to air dry. Repeat as needed.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use as Fungicides (Against Pathogenic Fungi): Effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 samples representing 2 different batches using the AOAC Fungicidal Test. Performance requirements for this test: the highest dilution that kills all fungal spores is the minimum effective concentration. Alternatively, the AOAC Use Dilution Method, modified to conform with appropriate elements in the AOAC Fungicidal Test, may be employed. If the product is intended for use as a spray, the AOAC Germicidal Spray Products Test must be employed. The inoculum in the above tests must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 samples representing 2 different batches must be employed in the test. Performance standard for this test: killing of the test microorganism on all carriers is required. These agency standards can be found in DIS/TSS-6.

Note: As an interim policy, the Agency is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes* and *Aspergillus niger*. The Agency recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until the Agency determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 474275-01 and 474844-05 "H-28504, H-28505, and H-28503: AOAC Germicidal Spray Test Using *Aspergillus niger*," by Felicia L. Sellers. Study conducted at Microbiotest, Inc. Study completion date – April 17, 2008. Study Identification Number 473-126.

This study was conducted against *Aspergillus niger* (ATCC 16404). Three lots (Lot Nos. H-28504, H-28505, and H-28503) of the product, Cryocide® Disinfectant Spray, were tested. The lot H-28503 was at least 60 days old. The test agent was received ready to use. In preparation of the inocula, the fungus was inoculated from the stock culture onto Sabouraud Dextrose Agar plates and incubated at 22–30°C for at least 10, but no more than 15 days or until sporulation occurred. When the cultures matured, the mycelial mats were removed from the surface of at least 5 plates and macerated in a sterile glass tissue grinder. Aliquots from selected dilution were plated on duplicate SDA plates. The plates were incubated for 2 days at 22-30°C. On the day of the test, suspensions were adjusted by dilution to yield approximately 3.3×10^7 conidia forming units/mL. Heat-inactivated horse serum was added to the inoculum to yield a 5% organic load. Ten (10) glass slide carriers per product lot were inoculated with 0.01-0.03 mL of a conidial suspension of the test organism. The carriers were dried for 20-40 minutes at 37±2°C. For each lot of product, carriers were sprayed with the product for 3-5 seconds at a distance of 6-8 inches from the carrier surface until thoroughly wet. Each carrier remained exposed to the product for 10 minutes at 25°C. Following exposure, the remaining liquid was drained off and carriers were transferred individually to tubes of 20 mL recovery broth with neutralizers and the tubes were then shaken. All tubes were then incubated at 22-30°C for up to 10 days but no more than 15 days. Controls included those for sterility, initial counts, viability, fungistasis and confirmation of challenge fungus. The reported average colony forming units per carrier, for the microorganism, is: ***Aspergillus niger* 1.4 x 10^6**.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: An amendment was submitted on July 31, 2008 to correct the carrier count unit from CFU/mL to CFU/carrier.

Note: The laboratory reports describe studies conducted for the product, Cryocide® Disinfectant Spray. Comparison of Cryocide® Disinfectant Spray's CSF, shown that it is 1:4 dilution of the product, Cryocide® 20, which is the subject of this efficacy report.

V. RESULTS

MRID # 474275-01 and 474844-05

Organism	Contact Time	No. Exhibiting Growth / Total No. Tested			Dried Carrier Count (CFU/carrier)
		Lot no. 51908A	Lot no. 51908B	Lot no. 41508A	
Aspergillus niger	10 minutes	0/10	0/10	0/10	1.4×10^6

VI. CONCLUSIONS

1. The submitted data (MRID 472152-01) **support** the use of the product, Cryocide® Disinfectant Spray (**1:4 dilution of Cryocide® 20**), as a disinfectant with fungicidal (specifically mold-killing) activity against ***Aspergillus niger*** (ATCC 16404) on hard, non-porous environmental surfaces at full strength (**or 1:4 dilution of Cryocide® 20**) and room temperature with a contact time of 10 minutes.

VII. RECOMMENDATIONS

1. The proposed label claims that the product, Cryocide® 20, **controls mold and mildew (*A. niger)*** on hard, non-porous environmental surfaces at **1:5 dilution** at room temperature with a contact time of 10 minutes **are not supported** by the applicant's data. **The only acceptable dilution rate is 1:4 (1 part of the concentrate Cryocide® 20 with 3 parts water)** for killing or control of mold and mildew.

2. The proposed label claims that the product, Cryocide® 20, can be used for **Preventive Maintenance Against Mold & Mildew at 1:8 dilution** are not acceptable. No data were for 1:8 dilution of the product. **The applicant must remove those claims**.

3. Animal viruses claims were made using 1:10 final dilution of Cryocide® 20 (acid-activated use solution). On previously accepted label, viruses were listed (Minute Virus of Mice, Mouse Hepatitis Virus, Rat Coronavirus, and Canine Parvovirus). Data provided by the applicant were generated using the product, Carnebon-200® EPA Reg. no. 9150-3, at 1:26 final dilution activated solution (100±5 ppm free chlorine dioxide). Minute Virus of Mice, Mouse Hepatitis Virus and Rat Coronavirus had 10 miniutes contact time; Canine Parvovirus, 15 minutes. **The applicant must list animal viruses and they respective contact time**.

4. The applicant must make the following changes to improve the proposed label:

- Under the "Preparation of Acid-Activated Use-Solution" section on page 4 of the proposed label, add, after 5 part of water, "(total 1 part Cryocide® 20 to 9 parts diluent)".

- Under the "Tuberculocidal (against *Mycobacterium bovis*, BCG)" section on page 4 of the proposed label, change ""(total 1 part Cryocide® 20 to **10 part** diluent)" to read "(total 1 part Cryocide® 20 to **9 parts** diluent)" and add **contact time of 10 minutes**.

- On page 5 of proposed label, change "1 part Cryocide® 20 into a clean pail and add **4 parts** water to make up your working solution" to read "1 part Cryocide® 20 into a clean pail and add **3 parts** water to make up your working solution", and **remove** "**For preventive maintenance against……..7 parts water to make your working solution**".

- Under the "Poultry Houses and Animal Confinement/ Research/ Laboratory Facilities" section on page 6 of the proposed label, list animal viruses and they respective contact time.

- Under the "Animal Transport Vehicles and Equipment" section and "Agricultural Storage Facilities" section on pages 6 and 7 of the proposed label, specify the quart of Cryocide® 20 to use: **Undiluted or diluted**.

- Under the "To Treat Surfaces Contaminated with Mold & Mildew" section on page 7 of the proposed label, change "Mix 1 part of the concentrate Cryocide® 20 with 4 parts water " to read "Mix 1 part of the concentrate Cryocide® 20 with 3 parts water ".

- On page 7 of the proposed label, **remove** the "For Preventive Maintenance against Mold & Mildew" section.